JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

New Insights into Controversies on the Antioxidant Potential of the Olive Oil Antioxidant Hydroxytyrosol

SASKIA J. RIETJENS,* AALT BAST, AND GUIDO R. M. M. HAENEN

Department of Pharmacology and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands

In the present study, the antioxidant profile of olive oil antioxidants was investigated. Hydroxytyrosol and oleuropein are potent scavengers of hydroxyl radicals ($OH^{\bullet}$), peroxynitrite (ONOOH), and superoxide radicals ($O_2^{\bullet-}$). Homovanillic alcohol, one of the main metabolites of hydroxytyrosol, and tyrosol are less potent scavengers of these reactive species. None of the olive oil antioxidants are good hypochlorous acid (HOCl) or hydrogen peroxide (H_2O_2) scavengers. Hydroxytyrosol efficiently protects against LDL oxidation *in vitro* and *in vivo*. However, no protective effect of hydroxytyrosol is usually demonstrated *ex vivo* against the oxidation of LDL isolated from humans after hydroxytyrosol consumption. The present study shows that this controversy is due to the isolation of LDL, which greatly reduces the protective effect of hydroxytyrosol against LDL oxidation. Hydroxytyrosol is an efficient scavenger of several free radicals. The physiological relevance of the high intrinsic antioxidant activity of hydroxytyrosol is illustrated by its protection against LDL oxidation.

KEYWORDS: Olive oil; hydroxytyrosol; oxidative stress; antioxidant; LDL

INTRODUCTION

The role of olive oil in the protection against cardiovascular disease has been highlighted in several studies (*1*−*5*). Olive oil, the main source of fat of the Mediterranean diet, is rich in oleic acid, a mono-unsaturated fatty acid. To date, most of the research on the protective effect of olive oil has been focused on its high mono-unsaturated fatty acid content.

Recent studies show that olive oil is more than just a mono-unsaturated fat. Olive oil is a natural product that contains a wide range of ingredients. The composition depends, among others, on the type of olives, the manufacturing process, and storage conditions. In general, extra virgin olive oil contains relatively high amounts of phenolic antioxidants (*6*), such as oleuropein, hydroxytyrosol, and tyrosol (**Figure 1**). These phenolic compounds have been shown to provide beneficial cardiovascular effects (*1*, *2*, *7*, *8*). Consequently, supplements containing phenolic antioxidants from olive oil with health-promoting claims have been developed.

The molecular mechanism of the cardiovascular effects of olive oil antioxidants has not been fully elucidated. Studies investigating the antioxidant effects of olive oil phenolic compounds are conflicting (*9*). It has been shown in both *in vitro* and human *in vivo* studies that phenolic compounds efficiently protect against LDL oxidation (*1*, *2*, *7*, *8*, *10*−*13*). However, no protective effect of hydroxytyrosol is usually found against LDL oxidation in *ex vivo* experiments (*14*−*17*). In these experiments, LDL is isolated from humans after hydroxytyrosol consumption, and subsequently, the oxidizability of this LDL is determined *ex vivo*.

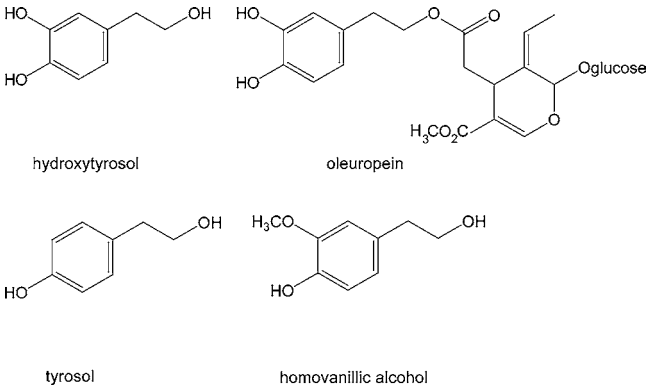

Figure 1. Structures of hydroxytyrosol, oleuropein, tyrosol, and homovanillic alcohol.

The present study is designed to provide new insights into the controversy on the effect of phenolic compounds on LDL oxidation. Some controversies also exist regarding the antioxidant profile of the phenolic compounds present in olive oil. For example, the reports on radical scavenging are ambiguous (*4*, *18*). Therefore, an integral antioxidant profile of hydroxytyrosol and related compounds is determined by quantifying their scavenging potential of $OH^{\bullet}$, ONOOH, $O_2^{\bullet-}$, HOCl, and H_2O_2. The intrinsic antioxidant activity forms the fundament for the physiological impact of olive oil phenolic compounds.

MATERIALS AND METHODS

Chemicals. Hydroxytyrosol was obtained from Cayman Chemical, Ann Arbor, MI. Oleuropein was obtained from Extrasynthese, Lyon, France. Tyrosol was purchased from Fluka, Buchs, Switzerland.

* To whom correspondence should be addressed. Tel: +31433881343. Fax: +31433884149. E-mail: S.Rietjens@farmaco.unimaas.nl.

10.1021/jf0706934 CCC: $37.00
Published on Web 08/07/2007

Dihydrorhodamine-123 (DHR-123), 2-deoxy-D-ribose, homovanillic alcohol, ascorbate, rutin, 2-methoxyphenol, phenol, HOCl, H_2O_2, dithionitrobenzoic acid, and mannitol were purchased from Sigma, St. Louis, MO. Catechol was obtained from Janssen Chimica, Geel, Belgium. Lipoic acid was purchased from Asta Medica AG, Frankfurt, Germany. Reduced glutathione (GSH) was obtained from ICN Biomedicals Inc., Costa Mesa, CA. Ebselen was obtained from Rhône-Poulenc Rorer, Köln, Germany. All other chemicals were of analytical grade purity.

Scavenging of Reactive Oxygen and Nitrogen Species. The antioxidant profile of hydroxytyrosol, oleuropein, tyrosol, and homovanillic alcohol was determined by quantifying their scavenging potential of reactive oxygen and nitrogen species. Scavenging of $OH^{\bullet}$ was determined by the deoxyribose method according to Halliwell et al. (*19*). Scavenging of $O_2^{\bullet -}$ was measured according to den Hartog et al. (*20*). The protection against ONOOH induced DHR-123 oxidation was measured as described by Kooy et al. (*21*). The HOCl scavenging potential of the compounds was measured according to Ching et al. (*22*). Scavenging of H_2O_2 was measured by adding 1 mM H_2O_2 to a 100 μM solution of the scavenger in a 150 mM sodium phosphate buffer (pH 7.4, 37 °C). UV spectra were recorded before and during 30 min after the addition of H_2O_2.

The reactive oxygen and nitrogen scavenging activities of the compounds tested were related to reference antioxidants that either have a high scavenging activity for one of these reactive species or were used for this purpose before. The reference compounds for $OH^{\bullet}$, ONOOH, $O_2^{\bullet -}$, HOCl, and H_2O_2 scavenging were, respectively, mannitol (*23*), ebselen (*24*), rutin, lipoic acid (*25*), and GSH.

LDL Isolation. Blood was collected from a healthy volunteer in tubes containing heparin and centrifuged at 3500 rpm at 4 °C for 10 min to obtain plasma. This study was performed in compliance with the guidelines of the Medical Ethical Review Board of the Academic Hospital, Maastricht, The Netherlands. Potassium bromide was added to plasma to increase its density (0.325 g per mL plasma). LDL was isolated by density gradient ultracentrifugation at 32,000 rpm at 4 °C for 17 h. The LDL fraction was isolated in a density range of 1.019−1.063 g NaCl/mL and diluted to a concentration of 1 mg/mL in a 50 mM sodium phosphate buffer at pH 7.4.

LDL Oxidation. LDL oxidation was performed at 37 °C in a 50 mM sodium phosphate buffer at pH 7.4 by adding 50 μM $CuSO_4$ to 50 μg/mL LDL in the presence of hydroxytyrosol or vehicle (ethanol). Conjugated diene formation was measured at 234 nm during 240 min.

The effect of the LDL isolation procedure on the protection by hydroxytyrosol against LDL oxidation was also determined. Hydroxytyrosol or vehicle (ethanol) was added to isolated LDL (1 mg/mL), vortexed, and incubated for 5 min. Subsequently, the LDL isolation procedure was repeated by creating the same density gradient. After ultracentrifugation, LDL was isolated and subsequently oxidized as described above. Mean lag times were calculated for all of the LDL experiments.

The addition of hydroxytyrosol (final concentration of 5 μM) to LDL that was isolated for a second time gave the same protection (data not shown) as hydroxytyrosol (5 μM) added to LDL after the first isolation procedure. This indicates that, with respect to the ability of hydroxytyrosol to protect, LDL has not been affected by the isolation procedure.

Lipophilicity. The lipophilicity of hydroxytyrosol was measured by determining the logarithm of the octanol water partitioning coefficient (log $P_{o/w}$). The octanol water partitioning coefficient ($P_{o/w}$) of hydroxytyrosol was calculated by dividing the concentration of hydroxytyrosol in the octanol layer by that in the aqueous layer (150 mM sodium phosphate buffer, pH 7.4) after mixing a 1 mM solution of hydroxytyrosol in buffer that was saturated with octanol with an equal volume of octanol that was saturated with buffer. The concentration of hydroxytyrosol in each layer was determined spectrophotometrically at 283 nm.

Statistics. The results are expressed as means ±SEM ($n = 3$). The LDL oxidation results are based on at least two separate experiments. For the results based on two experiments, the lag time is presented as mean ±half-range.

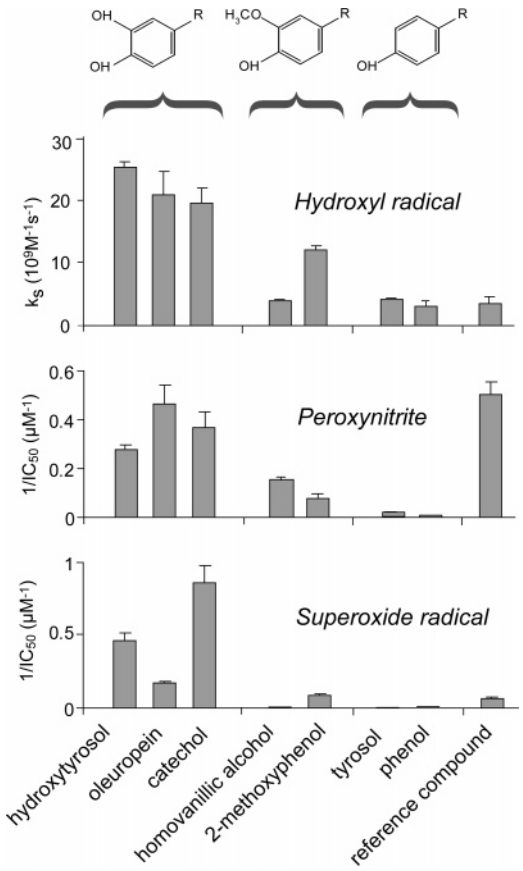

Figure 2. Antioxidant profiles of hydroxytyrosol, oleuropein, homovanillic alcohol, tyrosol, and related compounds. The $OH^{\bullet}$, ONOOH, and $O_2^{\bullet -}$ scavenging activities of the compounds were related to the scavenging activities of mannitol (second-order rate constant = $3.4 \cdot 10^9$ $M^{-1} \cdot s^{-1}$), ebselen (IC_{50} = 2.0 μM) and rutin (IC_{50} = 14.8 μM), respectively. The H_2O_2 and HOCl scavenging activity of the compounds was related to GSH (IC_{50} = 83 μM) and lipoic acid (second-order rate constant = 0.1 $mM^{-1} \cdot min^{-1}$), respectively. None of the phenolic compounds displayed any relevant H_2O_2 or HOCl scavenging activity. The H_2O_2 and HOCl scavenging activity of the reference compounds was at least 10 to 100 times higher than that of the phenolic compounds.

RESULTS

Both oleuropein and hydroxytyrosol were potent $OH^{\bullet}$, $O_2^{\bullet -}$, and ONOOH scavengers (**Figure 2**). Homovanillic alcohol, one of the main metabolites of hydroxytyrosol (**Figure 1**), was a relatively good scavenger of ONOOH and $OH^{\bullet}$. However, homovanillic alcohol was a less potent scavenger of $OH^{\bullet}$, $O_2^{\bullet -}$, and ONOOH than hydroxytyrosol. Compared to hydroxytyrosol, tyrosol was also a relatively poor scavenger of $OH^{\bullet}$, $O_2^{\bullet -}$, and ONOOH. None of the phenolic compounds from olive oil were good HOCl or H_2O_2 scavengers. The HOCl and H_2O_2 scavenging activity of the reference compounds was at least 10 to 100 times higher than that of the phenolic compounds.

To identify the functional group that is important in the antioxidant potential of compounds studied, the scavenging activities of catechol, 2-methoxyphenol, and phenol were also determined (**Figure 2**). Catechol was a potent scavenger of $OH^{\bullet}$, $O_2^{\bullet -}$, and ONOOH. Phenol was less potent in scavenging $OH^{\bullet}$, $O_2^{\bullet -}$, and ONOOH compared to catechol. The compound 2-methoxyphenol was a relatively good scavenger of $OH^{\bullet}$, $O_2^{\bullet -}$, and ONOOH, although the scavenging activities were lower than that of catechol. Catechol, 2-methoxyphenol, and phenol did not display any relevant H_2O_2 or HOCl scavenging activity.

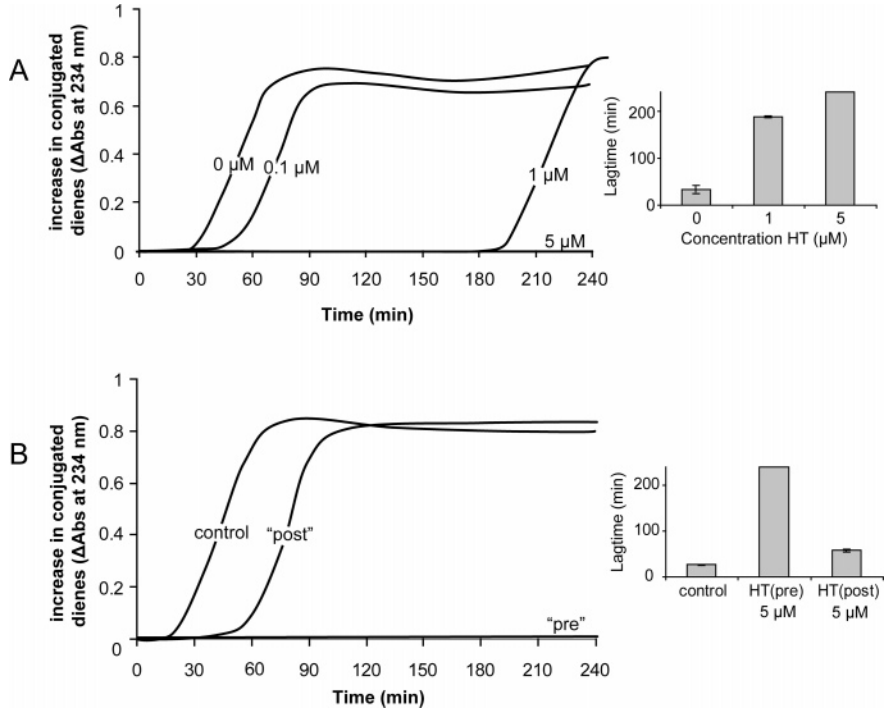

Figure 3. Protection by hydroxytyrosol (HT) against LDL oxidation. In panel **A**, the dose-dependent protection by hydroxytyrosol against LDL oxidation is shown. In panel **B**, the effect of the LDL isolation procedure on the protection by hydroxytyrosol against LDL oxidation is shown. Hydroxytyrosol is added to isolated LDL, and subsequently, the LDL isolation procedure is repeated. The protective effect of hydroxytyrosol before and after the second LDL isolation procedure is shown. The lag times of LDL oxidation are shown in the inserts. No conjugated diene formation occurred when a concentration of 5 μM hydroxytyrosol was used (before the second isolation procedure; panels **A** and **B**). For this reason, the lag time was set at 240 min, that is, the duration of the experiment.

Hydroxytyrosol dose dependently protected against LDL oxidation. A representative experiment is shown in **Figure 3**. When 5 μM hydroxytyrosol was added to LDL, the lag time in the *in vitro* assay was more than 240 min, whereas the lag time of the control experiment was 30 min (**Figure 3A**).

To examine the effect of the isolation of LDL, the isolation procedure was performed for a second time. Hydroxytyrosol (5 μM) was added to isolated LDL, and subsequently, the LDL isolation procedure was repeated for a second time. The protective effect of hydroxytyrosol against LDL oxidation was drastically reduced by the second isolation procedure. The lag time of oxidation was shortened from more than 240 min before the second LDL isolation procedure to 60 min after the second LDL isolation procedure (**Figure 3B**).

The lipophilicity of hydroxytyrosol, which was measured by determining the log $P_{o/w}$ value, was found to be 0.04. This is similar to the $P_{o/w}$ value reported previously (*26*). This means that the concentration of hydroxytyrosol in biological systems is expected to be very similar in the aqueous and lipid compartments.

DISCUSSION

The aim of the present study was to investigate the antioxidant potential of olive oil phenolic compounds. The fundament of the beneficial effect of an antioxidant is its intrinsic antioxidant activity. Therefore, the antioxidant profile of the olive oil antioxidants was established. Subsequently, the physiological impact of the antioxidant efficacy was evaluated by determining the protective effect against LDL oxidation.

In the present study, it was shown that both hydroxytyrosol and oleuropein have superior ONOOH, $OH^{\bullet}$, and $O_2^{\bullet-}$ scavenging activities, equal to or surpassing that of the reference antioxidants used. The high potency of hydroxytyrosol and oleuropein for scavenging ONOOH observed in the present study is in accordance with previous reports (*27*, *28*). Literature on the activity of hydroxytyrosol as an $O_2^{\bullet-}$ scavenger is contradictory (*4*, *18*). Our results indicate that hydroxytyrosol and oleuropein do scavenge $O_2^{\bullet-}$.

In the present study, it was shown that none of the olive oil phenolic compounds were good H_2O_2 or HOCl scavengers. Previous reports have suggested that hydroxytyrosol is able to scavenge H_2O_2 (*18*) and HOCl (*4*). The reported scavenging of H_2O_2 (*18*) by hydroxytyrosol is most likely the result of the scavenging of reactive species that are formed out of H_2O_2. In the only report on the HOCl scavenging of hydroxytyrosol, the activity of hydroxytyrosol was related to poor HOCl scavengers, that is, vitamin C and vitamin E. Comparing the activity of hydroxytyrosol to a more relevant reference compound clearly shows that hydroxytyrosol hardly possesses any HOCl scavenging activity. The scavenging of the non-radical species H_2O_2 and HOCl involves a two-electron reaction. In the scavenging of these species, thiols appear to be superior to phenolic compounds.

Our results show that compounds having a catechol (ortho-diphenolic) group in their molecular structure, that is, oleuropein and hydroxytyrosol, have superior antioxidant properties than compounds lacking this moiety, that is, homovanillic alcohol and tyrosol. As also reported previously, the present study shows that the antioxidant potency of the catechol group is superior to that of a resorcinol or phenol group. The relatively high antioxidant activity of catechol can be explained by the high electron donating effect of the second hydroxyl group (*29*, *30*).

For an antioxidant to have beneficial health effects *in vivo*, it is of course essential that it is taken up. Several studies in humans and rats indeed report that the uptake of hydroxytyrosol

Table 1. Overview of Human Studies on the Protective Effect of Olive Oil Phenolic Compounds against LDL Oxidation[a]

In Vitro

concentration hydroxytyrosol (μM)	effect	reference
0.1	+++	present study
1	++++	present study
5	++++	present study
0.3[b,c]	++	*12*
1.1[b,c]	+++	*12*
1.7[b,c]	++++	*12*
10[c]	+++	*10*
10[c,d]	++++	*11*

Ex Vivo

estimated daily intake (mg phenolic equivalents)			
olive oil	control	effect	reference
0.4	0	0	*14*
3.5[e]	0 and 1.6[e]	+	*2*
16[f]	0[f]	0	*15*
21	3	0	*17*
33	3	+[g,h]	*39*
100	0	0	*16*

In Vivo

estimated daily intake (mg phenolic equivalents)			
olive oil	control	effect	reference
3.5[e]	0 and 1.6[e]	+++	*2*
7.4[e]	0.7[e]	+	*7*
8.4[e]	0.1 and 3.7[e]	+++	*1*
11[e]	0.2 and 3[e]	+++	*8*
13.5[e]	0.1 and 6[e]	++	*13*

[a] The effect shown is based on the percentage increase in the lag time of conjugated diene formation in isolated LDL (*in vitro* and *ex vivo* studies) or the reduction in the extent of LDL oxidation measured using ELISA (*in vivo* studies). + corresponds with 0–10%, ++ corresponds with 10–30%, +++ corresponds with 30–100%, and ++++ corresponds with >100%. 0 means no effect. [b] The concentration of phenolic compounds present in olive oil was expressed as μM caffeic acid equivalents. [c] The protective effect was estimated from the figures presented the article. [d] The protective effect of oleuropein against the formation of thiobarbituric acid-reacting substances (TBARS) and lipid hydroperoxides was presented. [e] The daily intake was calculated using a density of olive oil of 0.92 kg/L. [f] Estimated phenol intake (according to Vissers et al., ref *9*). [g] Susceptibility of LDL to oxidation was assessed by the rate of TBARS formation. [h] Vitamin E intake was 12 vs 8 mg (high vs low phenolic equivalent diet).

is good (*31*−*34*). Plasma concentrations of hydroxytyrosol after consumption of 25 mL of extra virgin olive oil range from 50 to 160 nM (*8*, *35*). The consumption of supplements containing a relatively high amount of hydroxytyrosol might lead to an even higher hydroxytyrosol concentration. Hydroxytyrosol is metabolized in the body by the action of catechol-*O*-methyltransferase to homovanillic alcohol. Oleuropein itself hardly reaches the systemic circulation after ingestion. However, most of the oleuropein is hydrolyzed in the intestine, yielding hydroxytyrosol (*36*, *37*).

The physiological impact of the antioxidant efficacy of hydroxytyrosol was evaluated by determining its protective effect against LDL oxidation. LDL oxidation is one of the key steps in the initiation of atherosclerosis. The present study shows that hydroxytyrosol efficiently protects against LDL oxidation *in vitro* at relatively low concentrations. Review of the literature shows that our results are consistent with *in vitro* data from other studies on human LDL (**Table 1**). The present study also shows that the isolation procedure substantially reduces the protective effect of hydroxytyrosol against LDL oxidation. This might explain the false negative results in *ex vivo* studies that usually show no protective effect of olive oil phenolic compounds in LDL isolated from humans having consumed a diet rich in olive oil (**Table 1**).

During the isolation procedure, a relatively small volume of LDL is added to a relatively large volume of sodium chloride solutions. In the present study, it was shown that the log $P_{o/w}$ of hydroxytyrosol is 0.04. This indicates that the reduction in the protective effect might be due to the loss of hydroxytyrosol from LDL during the LDL isolation procedure. Recently, the loss of hydroxytyrosol from LDL during the isolation procedure has also been addressed by de la Torre-Carbot et al. (*38*). This is in line with the results of our study. The loss of antioxidants other than hydroxytyrosol during the isolation procedure might also be involved in the underestimation of the protective effect of hydroxytyrosol in *ex vivo* experiments. In addition to the lipophilicity, other properties also determine the interaction of antioxidants with LDL, such as hydrogen binding to proteins. Possible structural LDL changes during the isolation procedure may affect this interaction, which can also result in an underestimation of the protective effect of hydroxytyrosol in *ex vivo* experiments.

The *ex vivo* protective effect of olive oil phenolic compounds reported by Ramirez-Tortosa et al. (*39*) might be due to the higher vitamin E content in the olive oil diet compared to the control diet. Vitamin E, an extremely lipophilic compound, will not be removed from LDL during the isolation procedure. Marrugat et al. also demonstrated a protective effect of olive oil phenolic compounds against LDL oxidation, measured *ex vivo* after hydroxytyrosol consumption (*2*). However, the observed increase in lag time of LDL oxidation was only marginal.

LDL oxidizability has also been evaluated *in vivo* in several studies (**Table 1**). Both long and short term ingestion of olive oil rich in phenolic compounds have been shown to lower plasma levels of oxidized LDL (*1*, *2*, *7*, *8*). These effects are not attributable to the mono-unsaturated fatty acids of olive oil, as the fat compositions of the olive oil and the control oil were identical. These studies unequivocally proof that olive oil phenolic compounds provide protection against LDL oxidation.

In conclusion, hydroxytyrosol is an efficient scavenger of several free radicals. The physiological relevance of the high intrinsic antioxidant activity of hydroxytyrosol is illustrated by its protection against LDL oxidation.

ABBREVIATIONS USED

LDL, low-density lipoprotein; $OH^{\bullet}$, hydroxyl radical; ONOOH, peroxynitrite, $O_2^{\bullet -}$, superoxide radical; HOCl, hypochlorous acid; H_2O_2, hydrogen peroxide; $P_{o/w}$, octanol water partitioning coefficient; log $P_{o/w}$, logarithm of octanol water partitioning coefficient; GSH, reduced glutathione; TBARS, thiobarbituric acid reactive substances; DHR-123, dihydrorhodamine 123; SEM, standard error of the mean; ΔAbs, increase in absorbance; ELISA, Enzyme-Linked Immuno Sorbent Assay.

ACKNOWLEDGMENT

We would like to thank Marie-José Drittij, Marc Fischer, and Mathijs Groeneweg for their valuable assistance.

LITERATURE CITED

(1) Covas, M. I.; Nyyssonen, K.; Poulsen, H. E.; Kaikkonen, J.; Zunft, H. J.; Kiesewetter, H.; Gaddi, A.; de la Torre, R.; Mursu, J.; Baumler, H.; Nascetti, S.; Salonen, J. T.; Fito, M.; Virtanen, J.; Marrugat, J.; Group, E. S. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Ann. Intern. Med.* **2006**, *145*, 333−341.

(2) Marrugat, J.; Covas, M. I.; Fito, M.; Schroder, H.; Miro-Casas, E.; Gimeno, E.; Lopez-Sabater, M. C.; de la Torre, R.; Farre, M. Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation−a randomized controlled trial. *Eur. J. Nutr.* **2004**, *43*, 140−147.

(3) Rietjens, S. J.; Bast, A.; de Vente, J.; Haenen, G. R. The olive oil antioxidant hydroxytyrosol efficiently protects against the oxidative stress-induced impairment of the $NO^{\bullet}$ response of isolated rat aorta. *Am. J. Physiol. Heart Circ. Physiol.* **2007**, *292*, H1931−H1936.

(4) Visioli, F.; Bellomo, G.; Galli, C. Free radical-scavenging properties of olive oil polyphenols. *Biochem. Biophys. Res. Commun.* **1998**, *247*, 60−64.

(5) Visioli, F.; Bogani, P.; Grande, S.; Galli, C. Mediterranean food and health: building human evidence. *J. Physiol. Pharmacol* **2005**, *56*, 37−49.

(6) de la Torre-Carbot, K.; Jauregui, O.; Gimeno, E.; Castellote, A. I.; Lamuela-Raventos, R. M.; Lopez-Sabater, M. C. Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS. *J. Agric. Food Chem.* **2005**, *53*, 4331−4340.

(7) Fito, M.; Cladellas, M.; de la Torre, R.; Marti, J.; Alcantara, M.; Pujadas-Bastardes, M.; Marrugat, J.; Bruguera, J.; Lopez-Sabater, M. C.; Vila, J.; Covas, M. I. Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. *Atherosclerosis* **2005**, *181*, 149−158.

(8) Weinbrenner, T.; Fito, M.; de la Torre, R.; Saez, G. T.; Rijken, P.; Tormos, C.; Coolen, S.; Albaladejo, M. F.; Abanades, S.; Schroder, H.; Marrugat, J.; Covas, M. I. Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *J. Nutr.* **2004**, *134*, 2314−2321.

(9) Vissers, M. N.; Zock, P. L.; Katan, M. B. Bioavailability and antioxidant effects of olive oil phenols in humans: a review. *Eur. J. Clin. Nutr.* **2004**, *58*, 955−965.

(10) Visioli, F.; Bellomo, G.; Montedoro, G.; Galli, C. Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis* **1995**, *117*, 25−32.

(11) Visioli, F.; Galli, C. Oleuropein protects low density lipoprotein from oxidation. *Life Sci.* **1994**, *55*, 1965−1971.

(12) Fito, M.; Covas, M. I.; Lamuela Raventos, R. M.; Vila, J.; Torrents, L.; de la Torre, C.; Marrugat, J. Protective effect of olive oil and its phenolic compounds against low density lipoprotein oxidation. *Lipids* **2000**, *35*, 633−638.

(13) Covas, M. I.; de la Torre, K.; Farre-Albaladejo, M.; Kaikkonen, J.; Fito, M.; Lopez-Sabater, C.; Pujadas-Bastardes, M. A.; Joglar, J.; Weinbrenner, T.; Lamuela-Raventos, R. M.; de la Torre, R. Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. *Free Radical Biol. Med.* **2006**, *40*, 608−616.

(14) Bonanome, A.; Pagnan, A.; Caruso, D.; Toia, A.; Xamin, A.; Fedeli, E.; Berra, B.; Zamburlini, A.; Ursini, F.; Galli, G. Evidence of postprandial absorption of olive oil phenols in humans. *Nutr., Metab. Cardiovasc. Dis.* **2000**, *10*, 111−120.

(15) Nicolaiew, N.; Lemort, N.; Adorni, L.; Berra, B.; Montorfano, G.; Rapelli, S.; Cortesi, N.; Jacotot, B. Comparison between extra virgin olive oil and oleic acid rich sunflower oil: effects on postprandial lipemia and LDL susceptibility to oxidation. *Ann. Nutr. Metab.* **1998**, *42*, 251−260.

(16) Vissers, M. N.; Zock, P. L.; Leenen, R.; Roodenburg, A. J.; van Putte, K. P.; Katan, M. B. Effect of consumption of phenols from olives and extra virgin olive oil on LDL oxidizability in healthy humans. *Free Radical Res.* **2001**, *35*, 619−629.

(17) Vissers, M. N.; Zock, P. L.; Wiseman, S. A.; Meyboom, S.; Katan, M. B. Effect of phenol-rich extra virgin olive oil on markers of oxidation in healthy volunteers. *Eur. J. Clin. Nutr.* **2001**, *55*, 334−341.

(18) O'Dowd, Y.; Driss, F.; Dang, P. M.; Elbim, C.; Gougerot-Pocidalo, M. A.; Pasquier, C.; El-Benna, J. Antioxidant effect of hydroxytyrosol, a polyphenol from olive oil: scavenging of hydrogen peroxide but not superoxide anion produced by human neutrophils. *Biochem. Pharmacol.* **2004**, *68*, 2003−2008.

(19) Halliwell, B.; Gutteridge, J. M.; Aruoma, O. I. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem.* **1987**, *165*, 215−219.

(20) den Hartog, G. J.; Haenen, G. R.; Vegt, E.; van der Vijgh, W. J.; Bast, A. Superoxide dismutase: the balance between prevention and induction of oxidative damage. *Chem.-Biol. Interact.* **2003**, *145*, 33−39.

(21) Kooy, N. W.; Royall, J. A.; Ischiropoulos, H.; Beckman, J. S. Peroxynitrite-mediated oxidation of dihydrorhodamine 123. *Free Radical Biol. Med.* **1994**, *16*, 149−156.

(22) Ching, T. L.; de Jong, J.; Bast, A. A method for screening hypochlorous acid scavengers by inhibition of the oxidation of 5-thio-2-nitrobenzoic acid: application to anti-asthmatic drugs. *Anal. Biochem.* **1994**, *218*, 377−381.

(23) Haenen, G. R.; Jansen, F. P.; Bast, A. The antioxidant properties of five O-(b-hydroxyethyl)-rutosides of the flavonoid mixture venoruton. *Phlebology* **1993**, *8*, 10−17.

(24) Haenen, G. R.; Paquay, J. B.; Korthouwer, R. E.; Bast, A. Peroxynitrite scavenging by flavonoids. *Biochem. Biophys. Res. Commun.* **1997**, *236*, 591−593.

(25) Haenen, G. R.; Bast, A. Scavenging of hypochlorous acid by lipoic acid. *Biochem. Pharmacol.* **1991**, *42*, 2244−2246.

(26) Paiva Martins, F.; Gordon, M. H.; Gameiro, P. Activity and location of olive oil phenolic antioxidants in liposomes. *Chem. Phys. Lipids* **2003**, *124*, 23−36.

(27) Deiana, M.; Aruoma, O. I.; Bianchi, M. L.; Spencer, J. P.; Kaur, H.; Halliwell, B.; Aeschbach, R.; Banni, S.; Dessi, M. A.; Corongiu, F. P. Inhibition of peroxynitrite dependent DNA base modification and tyrosine nitration by the extra virgin olive oil-derived antioxidant hydroxytyrosol. *Free Radical Biol. Med.* **1999**, *26*, 762−769.

(28) de la Puerta, R.; Martinez Dominguez, M. E.; Ruiz Gutierrez, V.; Flavill, J. A.; Hoult, J. R. Effects of virgin olive oil phenolics on scavenging of reactive nitrogen species and upon nitrergic neurotransmission. *Life Sci.* **2001**, *69*, 1213−1222.

(29) Heijnen, C. G.; Haenen, G. R.; Vekemans, J. A.; Bast, A. Peroxynitrite scavenging of flavonoids: structure activity relationship. *Environ. Toxicol. Pharmacol.* **2001**, *10*, 199−206.

(30) Heijnen, C. G.; Haenen, G. R.; Oostveen, R. M.; Stalpers, E. M.; Bast, A. Protection of flavonoids against lipid peroxidation: the structure activity relationship revisited. *Free Radical Res.* **2002**, *36*, 575−581.

(31) Visioli, F.; Caruso, D.; Plasmati, E.; Patelli, R.; Mulinacci, N.; Romani, A.; Galli, G.; Galli, C. Hydroxytyrosol, as a component of olive mill waste water, is dose- dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Radical Res.* **2001**, *34*, 301−305.

(32) Visioli, F.; Galli, C.; Bornet, F.; Mattei, A.; Patelli, R.; Galli, G.; Caruso, D. Olive oil phenolics are dose-dependently absorbed in humans. *FEBS Lett.* **2000**, *468*, 159−160.

(33) Vissers, M. N.; Zock, P. L.; Roodenburg, A. J.; Leenen, R.; Katan, M. B. Olive oil phenols are absorbed in humans. *J. Nutr.* **2002**, *132*, 409−417.

(34) Tuck, K. L.; Freeman, M. P.; Hayball, P. J.; Stretch, G. L.; Stupans, I. The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. *J. Nutr.* **2001**, *131*, 1993−1996.

(35) Miro Casas, E.; Covas, M. I.; Farre, M.; Fito, M.; Ortuno, J.; Weinbrenner, T.; Roset, P.; de la Torre, R. Hydroxytyrosol disposition in humans. *Clin. Chem.* **2003**, *49*, 945−952.

(36) Edgecombe, S. C.; Stretch, G. L.; Hayball, P. J. Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. *J. Nutr.* **2000**, *130*, 2996−3002.

(37) Corona, G.; Tzounis, X.; Assunta Dessi, M.; Deiana, M.; Debnam, E. S.; Visioli, F.; Spencer, J. P. The fate of olive oil polyphenols in the gastrointestinal tract: implications of gastric and colonic microflora-dependent biotransformation. *Free Radical Res.* **2006**, *40*, 647−658.

(38) de la Torre-Carbot, K.; Chavez-Servin, J. L.; Jauregui, O.; Castellote, A. I.; Lamuela-Raventos, R. M.; Fito, M.; Covas, M. I.; Munoz-Aguayo, D.; Lopez-Sabater, M. C. Presence of virgin olive oil phenolic metabolites in human low density lipoprotein fraction: determination by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *Anal. Chim. Acta* **2007**, *583*, 402−410.

(39) Ramirez Tortosa, M. C.; Urbano, G.; Lopez Jurado, M.; Nestares, T.; Gomez, M. C.; Mir, A.; Ros, E.; Mataix, J.; Gil, A. Extra-virgin olive oil increases the resistance of LDL to oxidation more than refined olive oil in free-living men with peripheral vascular disease. *J. Nutr.* **1999**, *129*, 2177−2183.

Received for review March 9, 2007. Revised manuscript received June 18, 2007. Accepted June 21, 2007.

JF0706934